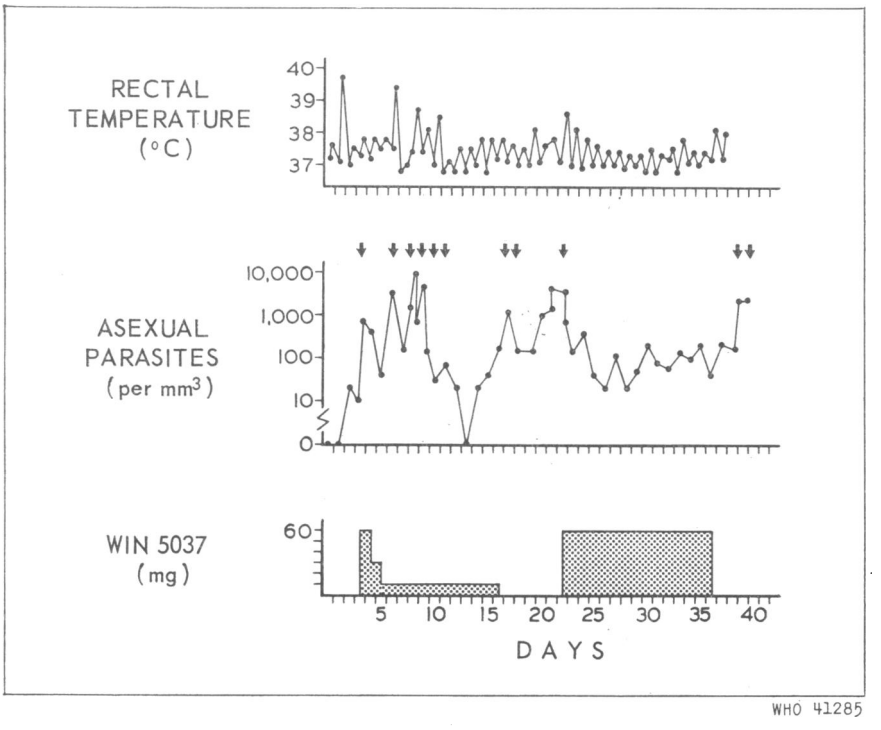
FIG. 2

COURSE OF TEMPERATURE AND PARASITAEMIA IN A SECOND VOLUNTEER HAVING A BLOOD-INDUCED INFECTION WITH A STRAIN OF CHLOROQUINE-RESISTANT *P. FALCIPARUM* FROM THAILAND [a]



[a] The volunteer (32 years old) weighed 200 pounds (90 kg). An infection with this strain of *P. falciparum* had been obtained by intravenous inoculation of a small sample of infected blood 20 days prior to day 1 of this study. A patent infection had been present for 16 days prior to day 1 of this study; the volunteer had received quinine intermittently during this time. Each black arrow designates the administration of 540 mg of quinine base orally.

Simian Malaria Parasites of Ceylon *

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In November 1963 true malaria parasites were reported from Ceylon monkeys for the first time.[a, b] Until then there were no authentic records of *Plasmodium* infection in local monkeys. Castellani & Chalmers [c] had reported "*P. kochi*", which caused illness and death of monkeys, and also noted that the bone marrow and spleen were pigmented. They added that the parasites were not inoculable to other monkeys. It is likely, therefore, that they

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[a] Dissanaike, A. S. (1963) *Trans. roy. Soc. trop. Med. Hyg.*, **57**, 488.

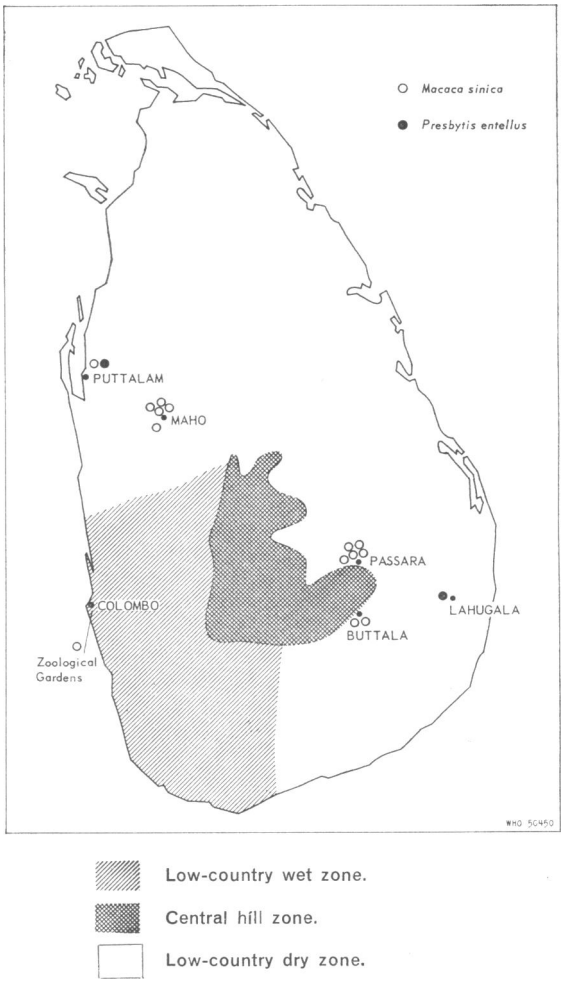
[b] Dissanaike, A. S. (1963) *Proc. Ceylon Ass. Advmt Sci.*, 19th session, p. 19 (abstract).

[c] Castellani, A. & Chalmers, A. J. (1910) *Manual of tropical medicine*, 1st ed., London.

were referring to a *Hepatocystis* or even a *Babesia* infection, but if it was the former, one cannot explain the illness and death of the animals. Crawford [d] reported malaria parasites from a monkey in Ceylon, but in a recent personal communication (1963) he states that this was a South American monkey referred to by Hill,[e] the parasite being probably *P. brasilianum*. A *Hepatocystis* infection was recorded from a local monkey recently by the present author.[f]

Three species of monkeys are found in Ceylon. The toque monkey, *Macaca sinica*, is similar to the South Indian bonnet monkey, *M radiata*, and is represented by two common subspecies, *M. sinica sinica* and *M. sinica aurifrons*. There are also two leaf monkeys, *Presbytis entellus thersites* (known as the grey langur), and *Presbytis senex* (black monkey) of which four subspecies have been recognized.[g] Ceylon has been divided into three main zones according to the distribution of mammals in general and these are based on climatic and physical conditions such as rainfall and altitude. In the low-country, dry-zone area, which occupies the greater part of the island (see the map), *Macaca sinica* and *Presbytis entellus* are found often in close association. *Presbytis senex*, on the other hand, is usually confined to the low-country wet-zone area, with one subspecies in the highlands and one in the low-country dry zone, while *Macaca sinica aurifrons* is distributed in the central hill zone and in the foothills of the low-country wet zone.

DISTRIBUTION OF MONKEYS FOUND WITH MALARIA PARASITES IN CEYLON



Material and methods

The animals studied were usually shot in the low-country dry-zone jungle areas. A blood film was taken; if that was positive, the heart blood was brought in ice to the laboratory and inoculated into laboratory toques which, wherever possible, had been previously splenectomized. Unfortunately only one rhesus monkey (M1) was available for inoculation. After inoculation, daily blood films were made at 4-6-hour intervals and these were stained in buffered Giemsa (3 drops of Giemsa to 1 ml of buffered distilled water) for 45 minutes and examined for parasites. Whenever an animal was trapped or captured blood films were first examined for several days and then the animal was splenectomized.

Anopheline larvae brought from an enzootic area were bred out to the adult stage and fed on positive monkeys with high gametocyte counts. Several wild-caught anophelines were dissected.

Results

In the present investigations 64 local monkeys were examined, of which 46 in the wild state. Of the latter, 14 out of 18 toques (*Macaca sinica*) and two out of 20 grey langurs (*Presbytis entellus thersites*) were infected. Table 1 summarizes the parasites recovered from these monkeys, while the accom-

[d] Crawford, M. (1937) In: *Administration report of the Acting Director of Agriculture for 1936*, Colombo, Ceylon Government Press.

[e] Hill, W. C. O. (1936) *J. comp. Path.*, **49**, 274.

[f] Dissanaike, A. S. (1963) *Ceylon vet. J.*, **11**, 73.

[g] Phillips, W. W. A. (1935) *Manual of the mammals of Ceylon*, Colombo, Colombo Museum.

TABLE 1
SUMMARY OF PLASMODIA RECOVERED FROM MONKEYS IN CEYLON

Simian host	Monkeys examined			*Plasmodium cynomolgi*	*Plasmodium shortti*	*Plasmodium* sp. nov.
	Total	In wild state	Positive			
Macaca sinica sinica	25	12	8	+	+	+
Macaca sinica aurifrons	7	6	6	+	+	+
Presbytis entellus	23	20	2	+	−	−
Presbytis senex	9	8	0	−	−	−
	64	46	16			

panying map shows the distribution of the positive animals. Table 2 indicates the isolations that have been made of parasites from monkeys that were shot or captured from various parts of the island. It is seen from these that *P. cynomolgi*-like parasites were recovered from *Macaca sinica* and *Presbytis entellus thersites*, while a *P. shortti*-like parasite and a "new" parasite were also isolated from *Macaca sinica*. These parasites are discussed in further detail below.

Plasmodium cynomolgi. As stated, *P. cynomolgi*-like parasites have been recovered from *Macaca sinica* and *Presbytis entellus*. Characteristic features are the Schüffner-type stippling, which becomes intense in the later stages and the enlargement of the erythrocytes. The maturation of schizonts takes place every third day between 9 and 11 a.m., the number of merozoites ranging from 12 to 18 with an average of about 16.

This parasite is seldom seen in blood films of wild monkeys and only appears in large numbers after splenectomy or after the blood from wild monkeys is inoculated into laboratory monkeys. One of the strains isolated was sent to Professor Garnham at the London School of Hygiene and Tropical Medicine, England, who has confirmed the identification but has observed peculiarities in its behaviour in mosquitos as well as in the rhesus monkey.

Plasmodium shortti. Blood from the first monkey that was shot was inoculated into a rhesus (M1) and a toque (M2). Both these animals became infected within eight to nine days and at first only *P. cynomolgi*-like parasites were present. This was confirmed by periodicity studies done about a fortnight after

TABLE 2
HISTORY OF ISOLATIONS OF PLASMODIA FROM LABORATORY MONKEYS

Source of monkey	Puttalam	Zoological Gardens (? origin)	Maho		Passara		
Original monkey shot or captured	M5	M11	M52	M53	M59	M61	M64
Parasites identified in blood film	*P. cynomolgi* *P. shortti*	*P. shortti*	*P. cynomolgi*	*P. cynomolgi*	Negative	*P. cynomolgi* *P. shortti*	*P. cynomolgi*
Blood inoculated to and/or splenectomized	M1 M2	M11 [a]	M18	M3	M59 [a]	M16 [a]	M15 [a]
			M18 [a]	M3 [a]			
Parasites finally isolated	*P. cynomolgi* *P. shortti*	*P. shortti* *P. cynomolgi*	*P. cynomolgi* P. sp. nov.	P. sp. nov. *P. cynomolgi*	*P. shortti* *P. cynomolgi*	*P. cynomolgi* P. sp. nov. *P. shortti*	*P. cynomolgi*

[a] Splenectomized.

they became positive. A few months later both animals showed much smaller parasites in slightly enlarged erythrocytes in which the stippling was more discrete and relatively scanty. The usual reddish background of the erythrocyte was also not seen. It was suspected that this parasite was *P. shortti*, and Professor Garnham confirmed this when he found two types of oocysts appearing in experimentally infected mosquitos and later isolated typical *P. shortti*-like parasites from rhesus monkeys. M1 and M2 were still showing *P. shortti*-like parasites (with fluctuations in the parasitaemia) 11 months after the initial inoculations. Since then two naturally infected toques (M11 and M59) had *P. shortti* as the predominant parasite, one of them (M59) becoming positive only after splenectomy. M59 was showing only *P. shortti* in large numbers, but died of an unexplained illness before a four- or eight-hourly periodicity chart could be prepared. Fortunately it was observed earlier that maturation of schizonts occurred just after noon and daily films taken at about this time before it died left little doubt as to its quartan periodicity. The merozoite numbers were between 9-16 with an average of about 12.

Plasmodium sp. nov. This parasite was first isolated from three monkeys from Maho in the North-Western Province of Ceylon. Blood examination of these monkeys showed only *P. cynomolgi*-like parasites, but a new parasite appeared after inoculation of blood into laboratory toques (M3, M4 and M18). *P. cynomolgi* and the new parasite appeared and disappeared from the peripheral blood at irregular intervals. M3 showed more parasites of the new type throughout, especially after it had been splenectomized. This parasite, when first seen, gave the impression of a degenerate organism in a distorted erythrocyte with an almost pathological formation of pigment in later stages of development. Blood films of M3 taken four-hourly at an early stage suggested a quotidian parasite and it was accordingly identified provisionally as *P. knowlesi edesoni*. The strain was sent to Professor Garnham, who agreed with this identification owing to the quotidian periodicity. It soon became evident that the quotidian periodicity noted earlier was due to two broods of parasites and that the periodicity was in fact tertian. Professor Garnham has since confirmed this and agrees that the parasite is different from the hitherto described species from monkeys.

Early ring stages of this parasite are delicate and assume all the forms described for similar parasites such as *P. k. edesoni* and *P. coatneyi*. Two, three and even four chromatin dots are common. Rings with double chromatin dots close together, at opposite poles and of equal or unequal size are frequent. *Accolé* and *appliqué* forms and later even band forms are seen stretching across the erythrocyte. Pigment can be made out at an early stage, appearing at first as a fine dust giving a brownish discoloration to the cytoplasm. The parasite now more or less disappears from the peripheral circulation, but one can usually see a few late trophozoites with increasing amounts of pigment. The parasites are ovoid and more or less compact but may take on varying shapes, while the pigment continues to increase in quantity and consistency, often becoming rod-like or like rice grains and later aggregating into larger masses. Sometimes the pigment may surround the chromatin, which is situated in a vacuole, while in other instances it may be clumped together at one or more points not infrequently separated from the rest of the parasite by a thin strand of cytoplasm.

Schizonts are particularly difficult to find in the peripheral blood, but may be seen between 11 a.m. and noon. The merozoites are generally few in number, between 4 and 10. Only one schizont was seen with as many as 14 merozoites. But in the heavy infections seen in the rhesus monkey, Professor Garnham has observed much larger numbers of merozoites. Female gametocytes are recognized by the larger amount of cytoplasm, with pigment in the form of large grains scattered within it. A few male gametocytes are seen with the usual diffuse chromatin and pinkish cytoplasm and are again characterized by abundant pigment.

Stippling appears to be of the Schüffner type, appearing in the late trophozoites as fine dots which become more prominent, though scanty, in the schizonts. By this time the red cell becomes more and more distorted, considerably shrunken and even fimbriated at times.

This parasite simulates *P. k. edesoni* (with which it was earlier confused), *P. coatneyi* and the new Nilgiri parasite of Eyles. The tertian periodicity distinguishes it from *P. k. edesoni*. It differs from *P. coatneyi* in the smaller number of merozoites, the appearance of the pigment and the absence of Maurer's clefts. Its affinities are mostly with the new Nilgiri parasite of Eyles,[h] from which it appears to be indistinguishable.

[h] Eyles, D. E. (1963) *J. Parasit.*, **49**, 866.

Mosquito studies. One of the enzootic foci (Passara), where six of seven toques examined were positive, was chosen for investigation of mosquito vectors. Collections of larvae were made from this area and the following adults were bred out and fed on monkeys when showing many gametocytes: *Anopheles maculatus*, *A. aconitus*, *A. vagus*, and *A. peditaeniatus*. Over 20 females of each of these species were examined after feeding on monkeys with the three different parasites but neither gut nor gland infections were observed.

Some preliminary attempts were made to trap mosquitos coming to feed on two sentinel monkeys (in Gater's trap) on three successive nights, once again in the same enzootic area. These collections were made bordering the jungle where the monkeys were believed to retire for the night. No attempts were made to go into the thick jungle or to make collections at canopy level. Once again the same species of anophelines were collected, but in addition there was one *A. nigerrimus*. None of these anophelines (53 in all) was infected, nor was any of the 53 *A. peditaeniatus* collected from another area (Galgamuwa).

No anophelines of the "*leucosphyrus*" group were collected, obviously since the catches were not made deep enough into the jungle or high enough at canopy level. This work is contemplated in the near future.

In this connexion it is of interest to note that the only record of malaria infection in wild anophelines (other than *A. culicifacies*) in Ceylon is that of Carter,[1] who reported two infections in *A. hyrcanus*. The parasites were not fully developed and the mosquitos not infective. The complexity of the "*hyrcanus*" group was not then appreciated and we do not know whether Carter's reference was to *A. peditaeniatus* or to *A. nigerrimus*. Our preliminary observations point to the latter, which may well prove to be the vector of simian malaria in Ceylon.

Blood inoculations into patients. Following several requests by surgeons (to infect patients suffering from thromboangiitis obliterans with malaria), we took the opportunity of testing the transmissibility of our parasites to man because the human parasites are no longer readily available in Ceylon. None of the four patients who were inoculated with blood from M1 (*P. cynomolgi*, *P. shortti*), M3 and M18 (*P. cynomolgi* and the new parasite) showed any parasites though they were examined for up to 30 days.

* * *

I wish to express my gratitude to Professor P. C. C. Garnham, F.R.S., for his valuable advice and opinions; to Dr R. A. Navaratne and Dr S. Nathkunam, who helped in splenectomizing the monkeys; to Mr Lyn de Alwis, Director of the Zoological Gardens, Colombo, for supplying us with monkeys from the reserve collection; to Mr F. P. de Alwis, Mr E. C. Hopman, Mr Zacky Dean, Mr Mervyn Lockhart, Mr L. C. R. de C. Wijetunge and Mr S. Nagamuttu, for shooting or capturing monkeys; to Mr W. J. Niles of the Medical Research Institute, Colombo, for assisting with the entomological investigations; and finally to Mr H. E. Bartholomeusz, Mr R. B. Arseculeratne, Mr M. P. A. Jayatillake, Mr S. Kumaranrajan and other members of the Department of Parasitology, University of Ceylon, for technical assistance at various times.

[1] Carter, H. F. (1944-45) *Trans. Soc. med. Off. Hlth Ceylon*, **11**, 24.